Claims:

1. Microorganism, characterized by the presence of a DNA sequence encoding a functional chaperone of a psychrophilic bacterium.

- 2. Microorganism according to claim 1, characterized in the DNA sequence encoding a functional chaperonin of a psychrophilic bacterium.
- 3. Microorganism according to claim 1, characterized in the DNA sequence encoding the chaperonin Cpn60 and/or Cpn 10 (SEQ ID No 1 and/or 2) of Oleispira antarctica.
- 4. Microorganism according to claim 1, characterized in the DNA sequence encoding a functional homolog of the chaperonin Cpn60 and/or Cpn10 of Oleispira antarctica (Seq ID Nr. 1 and/or 2) from a psychrophilic bacterium.
- 5. Microorganism according to claim 4, characterized in that the psychrophilic bacterium is selected from the group consisting of *Moraxella*, and *Alteromonas haloplanktis*.
- 6. Microorganism according to claim 1, characterized in the DNA sequence encoding a functional mutant of the chaperonin Cpn60 and/or Cpn 10 (Seq ID No 1 and/or 2) of Oleispira antarctica.
- 7. Microorganism according to claim 1, characterized in the DNA sequence encoding the stabilized single ring mutant chaperonin Glu461Ala/Ser463Ala/Val464Ala of Cpn60 (Seq ID No 11) or the mutant chaperonin Lys468Thr/Ser471Gly and/or Cpn 10.
- 8. Microorganism according to one of the preceding claims, which is selected among animal cell lines, plant cell lines, gram-positive or gram-negative bacteria, fungi and yeasts.
- 9. Microorganism according to one of the preceding claims, characterized in that the heterologous protein has enzymatic activity or hormonal activity in its native conformation.
- 10. Microorganism according to one of the preceding claims, characterized in that the DNA sequence encoding a functional chaperone is located chromosomally, extrachromosomally, or mitochondrially, or in chloroplasts of plants.
- 11. Process for producing a protein by heterologous expression in a host microorganism containing a gene sequence encoding the heterologous protein, characterized in that a microorganism according to one of the preceding claims is used.
- 12. Process according to claim 11, characterized in that the host organism is cultivated at a temperature below 25 °C, preferably 4 to 15 °C.

13. Process according to claim 11 or 12, characterized in that the heterologous protein is selected from the group consisting of mammalian proteins, psychrophilic mammalian or bacterial proteins, mesophilic bacterial, fungal or yeast proteins, and mutant or fusion variants thereof.

- 14. Process for changing the conformation of denatured proteins into their native and/or active conformation, characterized by the step of contacting the denatured protein with a functional chaperone of a psychrophilic bacterium.
- 15. Process according to claim 14, characterized in that the chaperone is the chaperonin Cpn60 and/or Cpn 10 (Seq ID No 1 and/or 2) of Oleispira antarctica in presence of at least one nucleotide, preferably adenosine triphosphate.
- 16. Process according to claim 11, characterized in that the chaperone is a functional homolog of the chaperonin Cpn60 and/or Cpn 10 (Seq ID No 1 and/or 2) from a psychrophilic bacterium or a functional mutant of the chaperonin Cpn60 and/or Cpn 10 (Seq ID No 1 and/or 2) of Oleispira antarctica.
- 17. Process according to one of claims 11 to 16, characterized in that the contacting is performed extracellularly or *in vitro*.
- 18. Process according to claim 17, characterized in that the contacting uses at least one immobilized chaperone.
- 19. Plant, characterized in that it can grow at lower ambient temperatures due to the presence of a DNA sequence encoding a cold-active functional chaperone of a psychrophilic bacterium or plant.
- 20. Plant according to claim 19, characterized in the DNA sequence encoding a functional chaperonin selected from the group consisting of Cpn60 and/or Cpn 10 (SEQ ID No 1 and/or 2) of Oleispira antarctica, a functional homolog thereof, and the stabilized single ring mutant chaperonin Glu461Ala/Ser463Ala/Val464Ala of Cpn60 (Seq ID No 11).

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEO ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTTATVLAQAIISEGLKSVAAGMN
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

Figure 2:

SEQ ID No 3: DNA coding for Cpn60 and Cpn10:

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

atcaaaaatgcagcaaggacagattcctgcccaagaattagcagaaggtttcttgttagcactggccggcgctttattattaacgccgg
gttttgtcactgatgcgctgggttttacattactcgtccccgcgacgcgtaaagcgttggtccataaggtgattgcatttattacccctc
gcatgatgactgcaagcagctttcaagcgacggtagttttcaggaaggctcgtttaaagatgtacattcgcacactgactcgcaaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaaagacgataagtaggtattttttcggctagccgttgaaatcctagtaaaagccc

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cgataaattaaccatctatttttcacagaggcaatttagcctttgtttaccttattgatcctaatacttgggatccaacagttggagagtctagc aaatgaaaatccgtccattacatgatcgtattgttgttcgccgtaaagaagaagaagaccgcaactgcgggtggtattattttacc gggcgctgcggcagaaaaaccaaatcaaggtgttgttatctctgtgggtactggccgtattcttgataatggttcagtgcaagcgctggc ggttaacgaaggcgatgttgtcgtttttggtaaatactcaggtcaaaatactatcgatatcgatggtgaagaattattgattttgaatga tattatttggtgatagcgcacgcgcaaaaatgttggtaggtgtaaacattttagccgacgcagtaagagttaccttaggacctaa aggtcgtaacgttgttatagaaaaatcatttggtgcaccgatcatcaccaaagatggtgtttctgttgcgcgtgaaatcgaattgaaagaca aattcgaaaacatgggcgcacagatggttaaggaagttgcttctcaagccaacgaccaagccggtgacggcacaacgacagcgact gtactagcacaggcgattatcagcgaaggcttgaaatctgttgcggctggcatgaatccaatggatcttaaacgtggtattgataaagcta ccgatgaaacggttggtcgtttaattgctgaagcgatggaaaaagtcggtaaagaaggtgtgattaccgttgaagaaggcaaaggcctt gaagacgagcttgatgttgtagaaggcatgcagttcgatcgcggttacttgtctccgtacttcatcaacaaccaagaaaaaatgaccgta gaaatggaaaatccattaattctattggttgataagaaaattgataaccttcaagagctgttgccaattcttgaaaacgtcgctaaatcaggt cgtccattattgatcgttgctgaagatgttgaaggccaagcactagcaacattggtagtaaacaacttgcgcggcacattcaaggttgc agcggttaaagcccctggttttggcgatcgtcgtaaagcgatgttgcaagatcttgccatcttgacgggtggtcaggttatttctgaagagtggcgcaggtactgaagcaagcgttaatactcgtgttgaccagatccgtgctgaaatcgaaagctcgacttctgattacgacatcgaaaa gttacaagaacgcgttgctaagcttgcgggcggcgttgccgtgattaaggttggtgcgggttctgaaatggaaatgaaagaagaaa gaccgtgttgacgatgcacttcatgcaactcgcgcagcggttgaagaaggtgttgttgcgggtggtggtgttgctttgattcgcgcactct cttcagtaaccgttgttggtgataacgaagatcaaaacgtcggtattgcattggcacttcgtgcgatggaagctcctatccgtcaaatcgc gggtaacgcaggtgctgaagggtcagtggttgttgataaagtgaaatctggcacaggtagctttggttttaacgccagcacaggtgagtatggcgatatgattgcgatgggtattttagaccctgcaaaagtcacgcgttcatctctacaagccgcggcgtctatcgcaggtttgatgat cacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgctggtggtatgcctgatatgggcggcatgggtggaatgggcg gtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgcttaccgtgtaaaaagatcaggctcaaggctgtctctataaaaag ccgtatctttgatgagtgttgtctttctgctgaaaacgacattcttggagtgcggctttttttgattttggtcataaaattcagaatattgtgtaatt ttatgtaactagetggeetataatgttgagtteetetgggtggeatgateteatggtaetteaettaageetgatteaetgeg gctttaacagtaaaataataacgcaacgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from Oleispira antarctica (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figur 4) 333 aa

MKNTLKSSSRFSLKQLGTGALIISSLFFGGCTTTQQDNLYTGVMSLARDSAGLEVKTA SAGDVNLTYMERQGSDKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG HGDSEQLLTTDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGGAISAIYSLSHPEKVKSL TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD VSAAAAFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of Oleispira antarctica (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *BamHI - Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

cgttattttattacacggtttctctgctgataaagataactggattctttttaccaaagaattcgatgaaaaatatcatgttatcgctgtcgattta gcgggacatggcgattcagaacaattattaacgactgattacggtctcataaaacaagccgagcgtttagatatcttcttatctggcttagg acattgatcgatgcagcaggtgtcgatggcgatactgaaagcgaatactacaaagttttggcagaaggtaagaatcctttaattgcaact gatgaagcaagttttgaataccgcatgggtttcaccatgactcagcctcctttcctaccttggccactaagaccttctttattacgtaaaacg ctagcccgtgccgagatcaataacaaaattttttccgatatgctgaaaaccaaagaacgtttaggaatgactaactttcaacagaaaattg aagtgaaaatggctcaacatccattgccaacactgattatgtggggcaaagaagatcgcgttcttgacgtatccgcagcagcggccttc ccaaattattcaacgaccaagctctgcggtaaaatcgcagtgggtttcttgttttcatcaacagcaacaaacgtgaaataccccgtaatcg catttttctgattatcaaaatacatactttccaccagcatattaacttcaacttttaaactcgtccgccctacctctataacactggcagtcaatt cgacaatggtacctgcgggaacaggatgcttaaaatcgattcgatcactgctgacggttacgatgctttgtcgagaaaaacgagtcgct gcaataaaagaaacctcatccatccactgcattgcagtgccaccgaataacgtatcatgatgatttgttgtctctggaaataccgctttaga ataataaatagttaacagtatattgaactgagggtctgaagaactctaatacctctgaagaactttgaggccgctagagagaaaagacca atatttcatatatatatttcacactacccttatctcactagacttcccgcgcataggcgcaaacaatcaacgcaagttcacaataaagcggttc gctgcaacacatgccctagcgtctaaagtagcacgcacaacactggccagtcgtactagcccctttgcgattcgtgcagacgagcaac aagcgctattaaacttacctaaatttctaaccaccaccattggttcttttccacaaaactcaaaaaactcgtcaaatccgcttgcaatttaaacg cgatgacatagatctaatcgattatcaaacccgcattcaagcgctcattaaaaacgcaccactggcaagaagttctacctgcactgacca gcaatcaaataaaaacgagttattgaggattttaattttaaaacaggtatattaataccctctctgtagtaaacaatgactgtatttacacaa aacttaggcattcaaattacagaaattggcgatgattatatcactggcacaatgccagcagatgcacgtaccttccagccaatgggactg attcatggcggctcaaatgtattgctggcagaaacactgggcagcatggcagctaactgctgtattaatttgtctcaagaatattgtgttgg ccaagaaattaacgccaaccacatacgcggtgttcgttccggcatagtgactggcacagcaacgctagtacacaaaggaagaacctc ccagatttgggaaattcgcatcgttaacgatccaaagaattcaaaaagcttctcgagagtacttctagagcggccgcggggcccatcgatt ticcacccgggtggggtaccaggtaagtgtacccaattcgccctatagtgagtcgtattacaattcactggccgtcgttttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonines with the esterase gene (EstRB8), all from Oleispira antarctica

SEO ID No 6: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 6) 97 aa:

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTTATVLAQAIISEGLKSVAAGMN
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEO ID No 8: estRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALIISSLFFGGCTTTQQDNLYTGVMSLARDSAGLEVKTA SAGDVNLTYMERQGSDKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG HGDSEQLLTTDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGGAISAIYSLSHPEKVKSL TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD VSAAAAFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with esterase of Oleispira antarctica (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by SacI site (pos. 69-75) and SalI site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

Small letters – the Cpn10-Cpn60 encoding fragment,

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGA ACAAAAGCTGGAGCTCctaatacttgggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgtatt gttgttcgccgtaaagaagaagaagaccgcaactgcgggtggtattattttaccgggcgctgcggcagaaaaaccaaatcaaggtgttgt tatctctgtgggtactggccgtattcttgataatggttcagtgcaagcgctggcggttaacgaaggcgatgttgtcgtttttggtaaatactc cttttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatttggtgatagcgcacgcgcaaaaatgttggtaggtgtaaacattttagccgacgcagtaagagttaccttaggacctaaaggtcgtaacgttgttatagaaaaatcatttggtgcaccgatcatcac caaagatggtgtttctgttgcgcgtgaaatcgaattgaaagacaaattcgaaaacatgggcgcacagatggttaaggaagttgcttctca agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaaatctgttgcgg gatacaaaagcaatcgctcaggtagggacaatctctgccaatgccgatgaaacggttggtcgtttaattgctgaagcgatggaaaaagt cggtaaagaaggtgtgattaccgttgaagaaggcaaaggccttgaagacgagcttgatgttgtagaaggcatgcagttcgatcgcggtt acttg tctccg tacttcatca acaacca agaaaaa atgaccg tagaaa tggaaaa tccatta attctattg gttgataagaaa attgataacaacattggtagtaaacaacttgcgcggcacattcaaggttgcagcggttaaagcccctggttttggcgatcgtcgtaaagcgatgttgca agatettgecatettgaeggtggteaggttatttetgaagagetagggatgtetttagaaactgeggateettettetttgggtaeggeaa

gttggtgcgggttctgaaatggaaatgaaagaagaagaagaccgtgttgacgatgcacttcatgcaactcgcgcagcggttgaagaag gtgttgttgcgggtggtggtgttgctttgattcgcgcactctcttcagtaaccgttgttggtgataacgaagatcaaaacgtcggtattgcat tggcacttcgtgcgatggaagctcctatccgtcaaatcgcgggtaacgcaggtgctgaagggtcagtggttgttgataaagtgaaatctg gcacaggtagctttggttttaacgccagcacaggtgagtatggcgatatgattgcgatgggtattttagaccctgcaaaagtcacgcgttc atctctacaagccgcggcgtctatcgcaggtttgatgatcacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgctggtg gtatgcctgatatgggcggcatgggtggaatgggcggtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgcttaccgt CAAACACCAATACCAATCGCAAAAACTCATAAAACTAGCCGATCACCAAATCCC AAAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGCTAA CGAACCAGGTCAAACTTATCGTTTTTTTGAGCACGTTTTGTTCCACTAATGAAAGA GAAAAGTCGTTAATTCACTGGCTTTTTGGCGTATCCGCACCTTCACATAGAAATTA GTAATGGCATGCTACTGGCCTTTAAAAAGAATCAGTTAATTGAAGAAACCTCGCT TATCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTAAA CTGACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAGAA TAATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACTCGGCAC CGGCGCTCTGATTATCTCCAGTTTGTTCTTCGGTGGTTGCACCACAACACAACAAG ATAATTTATACACAGGGGTTATGTCTCTTGCGAGAGACAGCGCTGGCCTAGAAGT TAAAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCAGT GACAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAAAG ATAACTGGATTCTTTTTACCAAAGAATTCGATGAAAAAATATCATGTTATCGCTGTC GATTTAGCGGGACATGGCGATTCAGAACAATTATTAACGACTGATTACGGTCTCA TAAAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTTAACTCATTT CACATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTGAGTC ACCCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATGGCG ATACTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAATTGC AACTGATGAAGCAAGTTTTGAATACCGCATGGGTTTCACCATGACTCAGCCTCCT TTCCTACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGCCGA GATCAATAACAAAATTTTTTCCGATATGCTGAAAAACCAAAGAACGTTTAGGAATG ACTAACTTCAACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAACAC TGATTATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGCCTT CAAAAAAATAATTCCACAAGCAACTGTTCATATTTTTCCTGAAGTAGGCCACCTA CCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGTCCT CTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTTAAA ATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCGGTAA

AATCGCAGTGGGTTTCTTGTTTTCATCAACAGCAACAAACGTGAAAATACCCCGTA ATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACTTCAAC TTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTCGACAATGGTAC CTGCGGGAACAGGATGCTTAAAATCGATTCGATCACTGCTGACGGTTACGATGCT GCAGTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAAATACCGCTTT AGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCTGCTAA ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAG GCCGCTAGAGAGAAAAGACCAGTGATAATATTTCATCTTGCCATGAGAGCTTATC ATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAA TACCAATATTCATATATAATTTCACACTACCCTTATCTCACTAGACTTCCCGC GCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTCGCTGCAACAC ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTT TGCGATTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACC ACCACCATTGGTTCTTTTCCACAAAACTCAAAAAACTCGTCAAAATCCGCTTGCAATT TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT AAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGC GGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGAAGGGAGCAGCAAAGAGGAAA ACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTTAAT TTTAAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTACAC AAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTG AAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAAT TACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACC TTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACAC TGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGG CCAAGAAATTAACGCCAACCACATACGCGGTGTTCCGTTCCGGCATAGTGACTGGC ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAAATTCGCATC GTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGG CCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira* antarctica (cpn10::stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala::est)

SEO ID No 10: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 8) 97 aa:

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

Below - Capital bold letters are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTTATVLAQAIISEGLKSVAAGMN
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEO ID No 12: EstRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGALIISSLFFGGCTTTQQDNLYTGVMSLARDSAGLEVKTA SAGDVNLTYMERQGSDKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG HGDSEQLLTTDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGGAISAIYSLSHPEKVKSL TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL

RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD VSAAAAFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the coexpression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from Oleispira antarctica (cpn10::stabilized single ring mutant

Glu461Ala/Ser463Ala/Val464Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by SacI site (pos. 69-75) and SalI site (pos. 2138-2143).

In the DNA sequence:

Small letters - the Cpn10-Cpn60 coding fragment,

Capital italics – fragments of vector

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

gatacaaaagcaatcgctcaggtagggacaatctctgccaatgccgatgaaacggttggtcgtttaattgctgaagcgatggaaaaagt cggtaaagaaggtgtgattaccgttgaagaaggcaaaggccttgaagacgagcttgatgttgtagaaggcatgcagttcgatcgcggtt acttgtctccgtacttcatcaacaaccaagaaaaatgaccgtagaaatggaaaatccattaattctattggttgataagaaaattgataac cttcaagagctgttgccaattcttgaaaacgtcgctaaatcaggtcgtccattattgatcgttgctgaagatgttgaaggccaagcactagc aacattggtagtaaacaacttgcgcggcacattcaaggttgcagcggttaaagcccctggttttggcgatcgtcgtaaagcgatgttgca agatcttgccatcttgacgggtggtcaggttatttctgaagagctagggatgtctttagaaaactgcggatccttcttctttgggtacggcaagttggtgcgggttctgaaatggaaatgaaagaagaaagaccgtgttgacgatgcacttcatgcaactcgcgcagcggttgaagaag gtgttgttgcgggtggtggtgttgctttgattcgcgcactctcttcagtaaccgttgttggtgataacgaagatcaaaacgtcggtattgcat tgg cact tcg tgg aagc tcc tatccg tcaa atcg cgg g taacg cag g tgctg Cag g G cag Cgg ttg ttg ataa ag tgaa at tag cact tcg tcg at tag can be a similar to the control of thectggcacaggtagctttggttttaacgccagcacaggtgagtatggcgatatgattgcgatgggtattttagaccctgcaaaagtcacgc gttcatctctacaagccgcggcgtctatcgcaggtttgatgatcacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgct ggtggtatgcctgatatgggcggcatgggtggaatgggcggtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgctta ccgtGTCGACATATTCAAGATAAAGATGCCTTCACTGACATCAGTCACCAACAATC AATCAAACACCAATACCAATCGCAAAAACTCATAAAACTAGCCGATCACCAAAT CCCAAAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGC TAACGAACCAGGTCAAACTTATCGTTTTTTTTGAGCACGTTTGTTCCACTAATGAAA GAGAAAAGTCGTTAATTCACTGGCTTTTTGGCGTATCCGCACCTTCACATAGAAAT TAGTAATGGCATGCTACTGGCCTTTAAAAAAGAATCAGTTAATTGAAGAAACCTCG CTTATCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTA AACTGACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAG AATAATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACTCGGC ACCGGCGCTCTGATTATCTCCAGTTTGTTCTTCGGTGGTTGCACCACAACACACA AGATAATTTATACACAGGGGTTATGTCTCTTGCGAGAGACAGCGCTGGCCTAGAA GTTAAAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCA GTGACAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAA AGATAACTGGATTCTTTTTACCAAAGAATTCGATGAAAAAATATCATGTTATCGCT GTCGATTTAGCGGGACATGGCGATTCAGAACAATTATTAACGACTGATTACGGTC TCATAAAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTTAACTC ATTTCACATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTG AGTCACCCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATG GCGATACTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAAT TGCAACTGATGAAGCAAGTTTTGAATACCGCATGGGTTTCACCATGACTCAGCCT

CCTTTCCTACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGC CGAGATCAATAACAAAATTTTTTCCGATATGCTGAAAAACCAAAGAACGTTTAGGA ATGACTAACTTCAACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAA CACTGATTATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGC CTTCAAAAAAAATAATTCCACAAGCAACTGTTCATATTTTTCCTGAAGTAGGCCAC CTACCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGT CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTT AAAATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCG GTAAAATCGCAGTGGGTTTCTTGTTTTCATCAACAGCAACAAACGTGAAATACCC CGTAATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACTT CAACTTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTCGACAATG GTACCTGCGGGAACAGGATGCTTAAAAATCGATTCGATCACTGCTGACGGTTACGA CATTGCAGTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACC GCTTTAGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCT GCTAAGAGTTGCGGATGGCATACATAAACTCGCTTGATTAAGATTAATAATAAAT AGTTAACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACT TTGAGGCCGCTAGAGAGAAAAGACCAGTGATAATATTTCATCTTGCCATGAGAGC AATAATACCAATATATTCATATATAATTTCACACTACCCTTATCTCACTAGACTT CCCGCGCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTCGCTGC AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC CCCTTTGCGATTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTC TAACCACCACCATTGGTTCTTTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTG CAATTTAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGC TCATTAAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAG CGGCGGCGAAGAGCTGCCTTTGATCGATCAAGAAGAAGAAGAGGGAGCAGCAAAGAGG AAAACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTT **AATTTTAAAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTA** CACAAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAG ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTC AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG TACCTTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAA ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTG

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751of Figure 10):

MKIRPLHDRIVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTTATVLAQAIISEGLKSVAAGMN
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAĖAMEKV
GKEGVITVEEGKGLEDELDVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala:

In the DNA sequence:
Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattcctgcccaagaattagcagaaggtttcttgttagcactggccggcgctttattattaacgccgg gttttgtcactgatgcgctgggttttacattactcgtccccgcgacgcgtaaagcgttggtccataaggtgattgcatttattacccctc gcatgatgactgcaagcagctttcaagcgacgggtagttttcaggaaggctcgtttaaagatgtacattcgcacactgactcgcaaagca gtcatgaaaaaatcacaattgaaggcgaatataccaaagacgataagtaggtattttttcggctagccgttgaaatcctagtaaaagccc cgataaattaaccatctatttttcacagaggcaatttagcctttgtttaccttattgatcctaatacttgggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgtattgttgttcgccgtaaagaagaagaagaccgcaactgcgggtggtattattttacc gggcgctgcggcagaaaaaccaaatcaaggtgttgttatctctgtgggtactggccgtattcttgataatggttcagtgcaagcgctggc ggttaacgaaggcgatgttgtcgtttttggtaaatactcaggtcaaaatactatcgatatcgatggtgaagaattattgattttgaatga tattatttggtgatagcgcacgcgcaaaaatgttggtaggtgtaaacattttagccgacgcagtaagagttaccttaggacctaa aggtcgtaacgttgttatagaaaaatcatttggtgcaccgatcatcaccaaagatggtgtttctgttgcgcgtgaaatcgaattgaaagaca aattcgaaaacatgggcgcacagatggttaaggaagttgcttctcaagccaacgaccaagccggtgacggcacaacgacagc gact gtactagcacaggcgattatcagcgaaggcttgaaatctgttgcggctggcatgaatccaatggatcttaaacgtggtattgataaagcta ccgatgaaacggttggtcgtttaattgctgaagcgatggaaaaagtcggtaaagaaggtgtgattaccgttgaagaaggcaaaggcctt gaagacgagcttgatgttgtagaaggcatgcagttcgatcgcggttacttgtctccgtacttcatcaacaaccaagaaaaaatgaccgta gaaatggaaaatccattaattctattggttgataagaaaattgataaccttcaagagctgttgccaattcttgaaaacgtcgctaaatcaggt cgtccattattgatcgttgctgaagatgttgaaggccaagcactagcaacattggtagtaaacaacttgcgcggcacattcaaggttgc agcggttaaagcccctggttttggcgatcgtcgtaaagcgatgttgcaagatcttgccatcttgacgggtggtcaggttatttctgaagagtggcgcaggtactgaagcaagcgttaatactcgtgttgaccagatccgtgctgaaatcgaaagctcgacttctgattacgacatcgaaaa gttacaagaacgcgttgctaagcttgcgggcggcgttgccgtgattaaggttggtgcgggttctgaaatggaaatgaaagaagaaa gaccgtgttgacgatgcacttcatgcaactcgcgcagcggttgaagaaggtgttgttgcgggtggtggtgttgctttgattcgcgcactct cttcagtaaccgttgttggtgataacgaagatcaaaacgtcggtattgcattggcacttcgtgcgatggaagctcctatccgtcaaatcgc

gggtaacgcaggtgctgCagggGcagCggttgttgataaagtgaaatctggcacaggtagctttggttttaacgccagcacaggtg agtatggcgatatgattgcgatgggtattttagaccctgcaaaagtcacgcgttcatctctacaagccgcggcgtctatcgcaggtttgat gatcacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgctggtggtatgcctgatatgggcggcatgggtggaatgggcgggtggaatgggcgggtggaatgggcgggtggaatgggcgggtgatgcctgtaaaaagatcaggctcaaggctgaaaaagacggtgtgtatttttgattttgattttggtcataaaaattcagaatattgtgtaatttttatgtaactagctggcctataatgttgagttcctctgggtggcatgatctcatggtacttcacttaagcctgattcactgcgggtttaacagtaaaataataagcgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc

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